Twin Zygosity Diagnosis by Mailed Questionnaire below Age Twelve Months

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Abstract. Parents of a sample of 76 same sexed pairs of twins aged 3 to 9 months completed a mailed similarity questionnaire. It included the Bonnelykke et al.'s questionnaire and a four anthropological variable scale. To improve each of these two methods, three other combined methods were carried out and results were compared with the biological zygosity diagnosis. The Bonnelykke et al.'s classification combined with anthropological scale (method 4) gave only 1.2% misclassified in the whole sample. It is concluded that zygosity diagnosis using this type of procedure to distinguish MZ and DZ pairs would be important not only for epidemiological study but also for pediatricians and parents.

Key words: Zygosity diagnosis, Questionnaire, Twins

INTRODUCTION

In 1979, Sandra Scarr and Louise Carter-Saltzman [13] used twins' responses to questions about their own, their parents, and others' judgements about their zygosity and physical similarity, and the ratings of similarity by eight judges, to estimate the perceived similarity of twins. The authors noted that twins, their parents, and others often make wrong judgments about twins' zygosity. According to Scarr and Carter-Saltzman (1979, p. 529), 18% to 40% of twins and their parents believed MZs to be DZs and the reverse. Raters who were asked to assign zygosity on the basis of photographs in large samples of twins had similarly high error rates.

However, in 1989, Bonnelykke et al. [4] showed that with their method only 5.3% subjects were misclassified and 0% unclassified. Bonnelykke et al. [4] proposed a questionnaire of zygosity to be filled out by parents of young twins aged 6 months to $6\frac{1}{2}$ years. Up to now this is the first study on young children. However, one can wonder if the power of classification would have the same validity on a population made up only by children aged 3 to 9 months. Despite the progress in molecular genetics and serological diagnosis [1-3, 5-7, 9, 11], zygosity diagnosis based on these last techniques is not commonly feasible at birth and investigations on zygosity either for epidemiological research and for pediatricians and parents remain important.

This study was undertaken to improve the validity of this similarity method in zygosity assessment of twin pairs under one year of age.

MATERIALS AND METHODS

This study focuses on twins born at the maternity unit of the Antoine Béclère Hospital, Clamart, France from August 1993 to July 1997. The parents were asked to fill out a twin similarity questionnaire sent by mail during the second month after birth. None of the parents knew the result of the diagnosis of zygosity. One hundred twenty-one questionnaires were completed and returned. Out of these, 36 twin pairs of unlike sex were not considered. Nine twin pairs were excluded because of unknown biological diagnosis of zygosity. The remaining 76 twin pairs (37 sets of boys and 39 sets of girls) were entered into the study. The questionnaires were filled out by parents between 3 and 9 months except one at 15 months (median = 130 days).

The diagnosis of zygosity was established using multiple red blood cell phenotypes (ABO, Rh, K, MNSs, Kidd, Duffy); the molecular biology techniques of amplifying DNA polymorphisms at five loci were used in a second step [12]. Monochorionic (MC) twins were classified as monozygotic (MZ). Among the dichorionic (DC) pairs, twins were considered to be dizygotic (DZ) if they showed differences in one or more serological markers. They were considered MZ if they were alike for all the markers (serological and DNA) used. All tests were performed in the Center of Perinatal Hemobiology, Saint Antoine Hospital, Paris, France.

The placental chorionicity was determined: 1) by lambda sign examination in ultrasound prediction during twin pregnancy [14, 15], and 2) by placental pathologic assessment at delivery. In case of disagreement between the sonographic and pathological diagnoses, the placental diagnosis analysis was kept.

The questionnaire was the same as Bonnelykke et al.'s (1989) but we added two anthropological items in part 3 "same shape of ear lobes" and "same shape of nose." We also added other questions concerning mixed identity in part 4 (items 9-11) "If yes, specify by the father, the mother, others," considering the age of the sample (3-15 months). Furthermore, in order to known parents' appreciation, but not aimed to serve to the diagnosis of zygosity, other questions have been set, such as "The further your children grow up, how do their similarities develop? (items 17-20 in part 7)" and "Do you think that it is important to know if twins are identical or fraternal: for the parents; for twins themselves (items 22-23 in the part 9)". Finally, the questionnaire included 26 questions grouped into 10 parts (see Appendix). All questions can be scored numerically.

The classification of zygosity according to the answers given in the questionnaire was made to test the accuracy of five different classification methods. First, the key deci-sion rules (method 1) for the questionnaire zygosity diagnosis are shown in Table 1. Dif-

Table 1 - Guidelines to the zygosity classification according to the questionnaire

	N	Quest. diagnosis	Question no		Number of misclassification
I	42	DZ	4-5	Different hair and/or eye color	3
II	3	DZ	1	No, not as two peas in a pod	0
			2	Ordinary family likeness	
			4-5	Same hair and eye color	
			8	No mixed identity	
			13	No external marks	
III	7	MZ	1	Yes, as two peas in a pod	0
			3	More than ordinary family likeness	
			4-5	Same hair and eye color	
			8	Mixed identity	
			13	Need for external marks	
IV	10	MZ	1	Yes, as two peas in a pod	0
			3	More than ordinary family likeness	
			4-5	Same hair and eye color	
			8	Mixed identity	
			13	No external marks	
V	2	MZ	1	No, as two peas in a pod	0
			3	More than ordinary family likeness	
			4-5	Same hair and eye color	
			8	Mixed identity	
			13	Need for external marks	
VI	5	MZ	1	No, as two peas in a pod	0
			3	More than ordinary family likeness	
			4-5	Same hair and eye color	
			8	Mixed identity	
			13	No external marks	
VII	7	XZ		Mixed answers - unclassified	
Total	76				3

Unclassified 7/76 = 9.2%

Misclassified 3/76 = 3.9%

ference with respect to hair and/or eye color was taken as proof of zygosity. In cases of identity of hair and eye color, description of more than ordinary family likeness and histories of mixed identity were considered indications of monozygosity. These rules are the same as those adopted by the Bonnelykke et al.'s study. We then improved a new classification (method 2) on the basis of the sole anthropological scale: "same hair, eye color, nose and ears". Subjects were classified on a scale from a score zero to four on the four anthropological items. For each item, a score of 1 was given if subjects were judged alike by parents, 0 corresponded to unlike parental judgement. The new key decision rules were so: score ≤2 confirm the fraternal (DZ) diagnosis and score >2 support the identical (MZ) diagnosis. Three other combined methods are presented too (methods 3, 4, 5).

RESULTS

The results of blood typing diagnosis showed 46 DZ sets and 30 MZ sets (22 monochorionic and 8 DC).

  The relation between answers to the individual questions and zygosity according to biological diagnosis is shown in Table 2, and the number of correctly classified twin pairs according to the individual questions is shown in Table 3. It should finally be mentioned that questions 2 and 3 "Family likeness" could classify 94.7% of the pairs correctly.

  Method 1: The Bonelykke et al.'s classification. According to this method when only the answers to the questionnaire were used, 3 of 76 pairs (4%) were misclassified, i.e., labeled in disagreement with the results of blood group determination. Mistakes were seen exclusively in MZ pairs (2 MC and 1 DC). Seven twin pairs of 76 (9%) were unclassified on the basis of the questionnaires when the rules described in Table 1 were followed. The answers were in all these ambiguous cases such as: "Not as two peas in a pod, only ordinary family likeness, same hair and eye color and mixed identity". Two of these needed (one MZ pair and one DZ pair), however, external marks. Three of these seven unclassifiable pairs were MZ according to the blood groups, the remaining were DZ.

  Method 2: Anthropological scale classification. With respect to these criteria (MZ >2, DZ <3 points), four of 76 pairs (5.3%) were misclassified. We found 32 MZ twin pairs and 44 DZ twin pairs including three and one misclassified, respectively. One MC-MZ misclassified as DZ, three DZ misclassified as MZ.

  Method 3: The Bonnelykke et al.'s classification improved by anthropological scale. To improve the power of classification of Bonnelykke et al.'s method (4% misclassified, 9% unclassified), we have classified these seven unclassified twin pairs (9%) by Bonnelykke et al.'s method, using anthropological scale. Following the criteria: score of 4 = MZ and score less than 4 = DZ, we found 3 MZ, 4 DZ and no misclassified subjects among these seven pairs.

  Method 4: Method 1 and 2 combined. Subjects were classified in such a manner that if a subject was classified identically into the two methods (method 1 and

Table 2 - Relation between answers to the individual questions and zygosity according to biological diagnosis

| Answers of the parents | Zygosity according to biological diagnosis | | | |
| | MZ | | DZ | |
	N	%	N	%
Questions for Zygosity Diagnosis				
1 Yes, as two peas in a pod	19	60	1	2
No, not as two peas in a pod	11	40	45	98
2 Ordinary family likeness	3	10	45	98
3 More than ordinary family likeness	27	90	1	2
4 Same hair	28	93	13	28
Different hair	2	7	33	72
5 Same eye color	28	93	18	39
Different eye color	2	7	28	61
6 Same shape of ear lobes	27	90	7	15
Different shape of ear lobes	3	10	39	85
7 Same shape of nose	29	97	12	26
Different shape of nose	1	3	34	74
8 Yes, mixed identity	28	93	20	44
No mixed identity	2	7	25	54
Unanswered			1	2
13 Need for external marks	9	30	1	2
No need for external marks	21	70	45	98
Parents' impression of zygosity				
14 "True"	16	53	0	
15 "False"	5	17	44	96
16 Don't know	9	30	2	4
Other Questions				
Parental perceptions of twin similarities or dissimilarities				
17 More and more similar	16	53	6	13
18 More and more dissimilar	6	20	14	30
19 Remained the same	7	23	3	7
20 Are as much dissimilar	1	3	23	50
Parental knowledge on the chorionicity				
21 Yes	13	45	26	57
No	16	55	20	43

(continued)

	Answers of the parents	Zygosity according to biological diagnosis			
		MZ		DZ	
		N	%	N	%
	Importance to know zygosity				
22	For the parents - Yes	24	80	37	80
	No	6	20	15	20
23	For twins themselves - Yes	23	77	36	78
	No	5	17	9	20
	Unanswered	2	6	1	2
	Who did fill out the questionnaire				
24	The mother - No	2	7	1	2
	Yes	28	93	45	98
25	The father - No	17	57	31	67
	Yes	13	43	15	33
26	Other - No	30	100	44	96
	Yes	0	0	2	4

Table 3 - Twin pairs correctly classified according to each individual questions

Question no		Correctly classified	
		N	%
1	As two peas in a pod	64	84.2
2	Family likeness	72	94.7
4-5	Hair and eye color	66	87
8	Mixed idetity	53	69.7
13	External marks	54	71.0
14	Parents' impression	60	78.9

method 2) leading to the correct classification, i.e., classified DZ or MZ according to the corroborating answers. We found 0% misclassified in comparison with the biological diagnosis of zygosity; 11.8 % (nine subjects) were unclassified since these twin pairs were classified in a different way into the method 1 and method 2. Subsequently, these nine unclassifiable subjects were classified on the anthropological scale respecting the method 3. Subjects were classified MZ when they had scored 4 points. Subjects were classified DZ when they had scored less than 4 points. We found three MZ pairs and 6 DZ pairs. Only one subject was misclassified, an MC-MZ labeled DZ in disagreement with the blood group determination. One of 76 (1.3%) was misclassified on the basis of this method 4. If the chorionicity was

known, subjects misclassified were therefore 0% taking into account that it was an MC-MZ who was misclassified.

Method 5: Classification on the basis of anthropological scale and "more than ordinary family likeness" item. This classification included two steps. First, screening of the MZ twins (anthropological scale = 4 points), then classification of subjects having less than 4 points by the answers to the item "more than ordinary family likeness". At the first step, there were 25 MZ classified and zero misclassification; at the second step we found 5 MZ and 46 DZ. One MZ (MC-MZ) and one DZ were misclassified (2.6%).

RESULTS FOR OTHER QUESTIONS

The parental perceptions of twin similarities or dissimilarities were studied with questions 17-20. The Chi Square on parental perceptions and biological zygosity diagnosis was significant (X2 = 34.79, DF = 3, P <0.00001). MZ twins were more alike to be judged as getting more and more similar, while DZ twins are judged as much dissimilar. But, this question is irrelevant for diagnosis classification (Table 2). Obviously, there would be a lot of misclassifications.

Concerning the analysis of questions at knowledge level on the chorionicity, at question 21 (part 8), "Did one give you information on the placenta (one or two sacs) at the maternity?", thirty-nine of 76 parents (51%) indicated "yes." We have verified if those who had a knowledge of placenta had a better impression as to zygosity. The impression of 87% of the 39 parents who had a knowledge of placenta was in line with the biological diagnosis. For those who had any placental information (36 of 76) in 72% of cases their impression was in line with the biological diagnosis. The difference between 87% and 72% was not significant.

At questions 22 and 23 (part 9) "Do you think it is important to know if twins are identical or fraternal (for the parents, for twins themselves)": 80% of the parents thought that it was important to know the zygosity for themselves; 77% of the parents thought it was important for twins, 17% thought it was not important and 6% did not answer.

Questions 24 to 26 (part 10) indicated that the questionnaire was filled out by both mother and father (34%), the mother alone (62%) and the father alone (2.6%). In brief, the mother participated to fill out the questionnaire for all subjects except three.

DISCUSSION

Results of the Bonnelykke et al.'s study found 5% unclassifiable subjects and 4% misclassified, following their method, the present study found 9.2% and 3.9% respectively. These differences are not significantly. However, we can think that this slight difference is due to the younger age of our sample. Indeed, it can be for example more difficult for the parents to precise the hair color when there is no much hair.

We have shown that with another method, including four anthropological variables instead of two, only 5.3% of subjects were misclassified and 0% unclassified. This method, based on a four-point scale, is quick, simple and has no need for computer.

To improve each of these methods, two combined methods were carried out. The first combination (method 3) classified the unclassified subjects of the Bonnelykke et al.'s method using our scale with the break point <3 = DZ, and ≥ 3 = MZ. So all subjects were classified and there was only 4% misclassified in the whole sample. Another combined method (method 4), slightly longer to perform, was realized giving a rate of 1.2% misclassified. This rate would be 0% when the chorionicity is known. The method 5, using two items of our questionnaire, was less accurate than method 4. Therefore, we think that method 4, which requires agreement between the Bonnelykke et al.'s method and the anthropological scale, is the best.

Ooki et al. (1993) [10] have carried out a zygosity questionnaire for populations (e.g., Japanese) where physical characteristics have no value because they have less variation than Caucasian. Following the same way, a 0-4 point scale can be carried out on the basis of our questionnaire and data. One point is scored for a yes at items 1, 3, 9 or 10, 14, but zero point if the answer is no. Subjects are classified MZ if the total score is more than one and DZ if it is less than two. Using their method without anthropological scale all DZ have the correct zygosity diagnosis but 3 from 30 MZ (2 MC-MZ and 1 DC-MZ) are misclassified. Therefore, on the whole sample there is 4% of misclassification. This rate is not significantly different from the rate of Ooki et al.'s study (7%).

In no study using similarity questionnaire the knowledge of placenta type by parents or twins has been verified. In the present study, we have made an estimation of this knowledge and although it seemed to improve the diagnosis, it was not significant. We cannot assert that this question biased data of the present study.

It should be noted that 80% of the parents thought that it was important for them to know the twin zygosity. In the same way Machin stated (1994) [8]: "For reasons of personal rights to identity, medico-legal responsibility, potential for transplantation, early education, and concordance/discordance for genetic disease, many feel that routine determination of twin zygosity at birth is needed and should be implemented to in the near future. Apart from the benefits to the twins and their parents, the consequences for research in twins and genetics would be enormous, since there remain many puzzling aspects of twinning". Therefore, it is important to continue research on zygosity diagnosis using biological markers and questionnaire.

It is obvious that these studies would have been duplicated by others laboratories for confirmation. If results were similar, method 4 would be highly accurate, quick and free and its use would be important not only for epidemiological study but also for pediatricians and parents.

It should be noted that also for Asian population it will be necessary to find physical characteristics having relevant variation.

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Availability

The French adaptation of the questionnaire is available upon request to the last author.

APPENDIX: TWIN SIMILARITY QUESTIONNAIRE

Part 1 -   Are the twins much alike in general appearance (as two peas in a pod)?

      (1) Yes _   No _

Part 2 -   Is the likeness between the twin as
          (2) Ordinary family likeness?   –
          (3) More than ordinary family likeness (remarkably alike)   –

Part 3 -   Do twins have the
          (4) Same hair color   –
          (5) Same eye color   –
          (6) Same shape of ear lobes   –
          (7) Same shape of nose   –

Part 4 -   Have the twins been mixed up by family or friends?
          (8) Yes_   No
          If Yes, specify:
           (9) The father
        (10) The mother
        (11) Other members of your family

      -   Have the twins been mixed up by people no relative of yours?
        (12) Yes _   No _

Part 5 -   Have the twins ever been so much alike that external marks have been needed
          to tell them apart?
        (13) Yes _   No_

Part 6 -   Your good self do you think so they are:
        (14) Right
        (15) Wrong
        (16) Don't know

Part 7 -   As your twins have grown older, has their similarity
        (17) become more and more similar
        (18) become more and more dissimilar
        (19) remained the same
        (20) remained as much dissimilar

Part 8 -   Did one give you information on the placenta (one or two sacs) at the maternity?
        (21) Yes _ No _

Part 9 -   Do you think it is important to know if twins are identical or fraternal?
        (22) for the parents   Yes _   No
        (23) for the twins themselves   Yes   No _

Part 10 - Who did fill out the questionnaire?
        (24) The mother   Yes _   No _
        (25) The father   Yes   No_
        (26) Other   Yes_   No_